

PENDING CLAIMS

5. (Amended) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprises:

a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

- i) an upstream universal priming site (UUP); and
- ii) a first target-specific sequence comprising a first base at an interrogation position; and

b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:

- i) a downstream universal priming site (DUP); and
- ii) a second target-specific sequence;

wherein if said first base is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed wherein at least one of said first and second ligation probes comprises an adapter sequence;

- c) removing non-hybridized probes;
- d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- e) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;
- f) contacting said amplicons with an array of capture probes; and
- g) determining the nucleotide at said detection position.

9. (Amended) A method according to claim 5, 26, 32 and 33 wherein said removing comprises:

- a) enzymatically adding a binding ligand to said target sequence to form a target sequence comprising said binding ligand;
- b) binding a hybridization complex comprising said target sequence comprising said binding ligand to a binding partner immobilized on a solid support;
- c) washing away unhybridized probes; and
- d) eluting said probes off said solid support.

10. A method according to claim 5, 26, 32, or 33 wherein said removing is done using a double-stranded specific moiety.
11. A method according to claim 10 wherein said double-stranded specific moiety is an intercalator attached to a support.
12. A method according to claim 11 wherein said support is a bead.
13. A method according to claim 5, 26, 32, or 33 wherein said amplifying is done by:
 - a) hybridizing a first universal primer to said UUP;
 - b) providing a polymerase and dNTPs such that said first universal primer is extended;
 - c) hybridizing a second universal primer to said DUP;
 - d) providing a polymerase and dNTPs such that said second universal primer is extended;and
 - e) repeating steps a) through d).
14. A method according to claim 5, 26, 32, or 33 wherein said array comprises:
 - a) a substrate with a patterned surface comprising discrete sites; and
 - b) a population of microspheres comprising at least a first subpopulation comprising a first capture probe and a second subpopulation comprising a second capture probe.
15. A method according to claim 14 wherein said discrete sites comprise wells.
16. A method according to claim 14 wherein said substrate comprises a fiber optic bundle.
19. (Amended) A method according to claim 5 or 32, further comprising providing a support on which the target sequence is immobilized.
20. (Amended) A method according to claim 19, wherein said non-hybridized probes are removed without removing said target sequence from said support.
21. (Amended) A method according to claim 5 or 32, further comprising attaching said target sequence to a support.
22. (Amended) A method according to claim 21, wherein said target sequence is attached to said support by a method selected from the group consisting of labeling said target sequence with a functional attachment moiety that binds said support, absorption of said target sequence on a charged support, direct chemical attachment of said target sequence to said support and photocrosslinking said target sequence to said support.
23. (Amended) A method according to claim 9, wherein said support is selected from the group consisting of paper, plastic and tubes.

26. (Amended) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain adjacent to said detection position and a second target domain comprising said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:
 - i) an upstream universal priming site (UUP); and
 - ii) a first target-specific sequence comprising a first base at an interrogation position; and
- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence;

wherein if said first base is perfectly complementary to said nucleotide at said detection position a ligation complex is formed and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- d) removing non-hybridized probes;
- e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- f) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;
- g) contacting said amplicons with an array of capture probes; and
- h) determining the nucleotide at said detection position.

30. (New) A method according to claim 9 wherein said solid support is a bead.

31. (New) A method according to claim 26 wherein said non-hybridized probes are removed without removing said target sequence from said support.

32. (New) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:

- i) an upstream universal priming site (UUP);
 - ii) a first target-specific sequence; and
 - iii) an interrogation position that is complementary to said detection position; and
 - b) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence;
- whereby if said interrogation position of said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, wherein at least one of said first and second ligation probes comprises an adapter sequence;
- c) removing non-hybridized probes;
 - d) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
 - e) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;
 - f) contacting said amplicons with an array of capture probes; and
 - g) determining the nucleotide at said detection position.

33. (New) A method of determining the identification of a nucleotide at a detection position in a target sequence comprising a first target domain comprising said detection position and a second target domain adjacent to said detection position, wherein said method comprises:

- a) providing a support on which the target sequence is immobilized;
- b) hybridizing a first ligation probe to said first target domain, said first ligation probe comprising:
 - i) an upstream universal priming site (UUP);
 - ii) a first target-specific sequence; and
 - iii) an interrogation position; and
- c) hybridizing a second ligation probe to said second target domain, said second ligation probe comprising:
 - i) a downstream universal priming site (DUP); and
 - ii) a second target-specific sequence;

whereby if said interrogation position of said first probe is perfectly complementary to said nucleotide at said detection position, a ligation complex is formed, and wherein at least one of said first and second ligation probes comprises an adapter sequence;

- d) removing non-hybridized probes;
- e) providing a ligase that ligates said first and second ligation probes to form a ligated probe;
- f) amplifying said ligated probe using said UUP and said DUP to generate a plurality of amplicons;
- g) contacting said amplicons with an array of capture probes; and
- h) determining the nucleotide at said detection position.

34. (New) A method according to claim 15, wherein said substrate comprises a fiber optic bundle.